

## Electronic Supplementary Information

### **Amplified fluorescence detection of T4 polynucleotide kinase activity based on coupled Exonuclease III reaction and graphene oxide platform**

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## Experimental section

### Reagents and apparatus

ExoIII (100 U  $\mu\text{L}^{-1}$ ), T4 PNK (10 U  $\mu\text{L}^{-1}$ ), T4 DNA Ligase (400 U  $\mu\text{L}^{-1}$ ), Dam (8 U  $\mu\text{L}^{-1}$ ) were purchased from New England Biolabs Ltd. (Beijing). ALP (EC 3.1.3.1) was purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Bovine serum albumin (BSA), human serum albumin (HSA), were purchased from Dingguo Biotech. Co. (Beijing, China). Adenosine diphosphate (ADP) and ammonium sulfate (( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub>) were purchased from Sigma Co., Ltd. (St. Louis, MO, USA). Ethylenediaminetetraacetic acid sodium (EDTA) and disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>) were purchased from Shanghai Chemical Reagents (Shanghai). GO was bought from Leadernano Co. Ltd. (Jining, China) and used without further purification. The buffers for enzyme reactions involved in this work were T4 DNA ligase buffer (66 mM Tris-HCl, 6.6 mM MgCl<sub>2</sub>, 10 mM DDT, 100 mM ATP, pH 7.6), the detection buffers contained 100 mM NaCl and 25 mM HEPES (pH 7.4). All solutions were prepared with Milli-Q water (resistance >18 M $\Omega$ ·cm) from a Millipore system. DNA oligonucleotides used in this work were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai), and their sequences are shown as follows:

P1: 5'-PO<sub>4</sub>-TAC AAG CG-3'

P2: 5'-AGT CGA GGA GAT-3'

P3: 5'-/FAM/-CCT CGA CTC GCT TGTA-3'

P4: 5'-TAC AAG CG-3'

P5: 5'- PO<sub>4</sub>-AGT CGA GGA GAT-3'

All fluorescence measurements were carried out on an F-4600 spectrometer (Hitachi, Japan). The instrument settings were chosen as follows:  $\lambda_{\text{ex}}$ =494 nm (slit 5 nm),  $\lambda_{\text{em}}$ =518 nm (slit 5 nm), PMT detector voltage=950 V.

### Optimization of GO concentration

To investigate the effect of GO concentration for fluorescence quenching, different concentrations of GO were mixed with 50 nM P3 in 80  $\mu\text{L}$  HEPES buffer (100 mM NaCl, 25 mM HEPES, pH 7.4) for 15 min at 37 °C, then the fluorescence changes were recorded.

### **Amplified assay of T4 PNK activity**

To investigate the activity of T4 PNK, samples were first prepared with 200 nM P1, 200 nM P2 and 200 nM P3, T4 PNK at various concentrations and T4 DNA ligase (20 U) was added into 19  $\mu\text{L}$  of  $1 \times$  T4 DNA ligase buffer (66 mM Tris-HCl, 6.6 mM  $\text{MgCl}_2$ , 10 mM DDT, 100 mM ATP, pH 7.6) for 30 min at 37  $^\circ\text{C}$ . Then, 1  $\mu\text{L}$  ExoIII (2 U  $\mu\text{L}^{-1}$ ) was introduced into the mixture immediately for 30 min at 37  $^\circ\text{C}$ . Finally, the reaction mixture was added of 8  $\mu\text{L}$  GO (200  $\mu\text{g mL}^{-1}$ ) and 52  $\mu\text{L}$  detection buffer (100 mM NaCl, 25 mM HEPES, pH 7.4) to keep the final volume reach 80  $\mu\text{L}$ , the solutions were incubated at 37  $^\circ\text{C}$  for 15 min prior to fluorescence measurement.

### **Detection of the inhibition of T4 PNK**

In the inhibition assay, ADP (0 ~ 10 mM),  $(\text{NH}_4)_2\text{SO}_4$  (0 ~ 60 mM),  $\text{Na}_2\text{HPO}_4$  (0 ~ 60 mM) and EDTA (0 ~ 60 mM) were selected as four kinds of inhibitors to investigate the effects of inhibitors on the PNK-catalyzed phosphorylation process. The experiments were performed in the typical reaction buffer which contained 200 nM P1, 200 nM P2 and 200 nM P3, 0.5 U  $\text{mL}^{-1}$  T4 PNK and T4 DNA ligase 1 U  $\mu\text{L}^{-1}$ . The reaction and measurement procedures were same to the above activity assays.

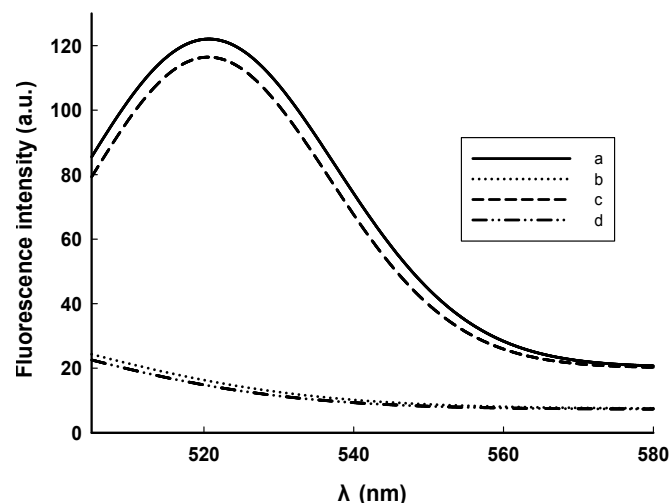
### **T4 PNK activity detection in diluted cell extracts**

A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U  $\text{mL}^{-1}$  penicillin, 100  $\mu\text{g mL}^{-1}$  streptomycin at 37  $^\circ\text{C}$  under 5% humidified  $\text{CO}_2$ . Cell extracts were obtained from  $1 \times 10^6$  cells which prepared according to the previous reports.<sup>1,2</sup> For the T4 PNK activity detection in cell extracts, 1% (v/v) cell extracts was added in the reaction buffer with all the other conditions the same as the description in the aforementioned experiment in pure buffer solution. All experiments were performed in compliance with the relevant laws and institutional guidelines of the Qufu Normal University hospital.

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## Supplementary Figures



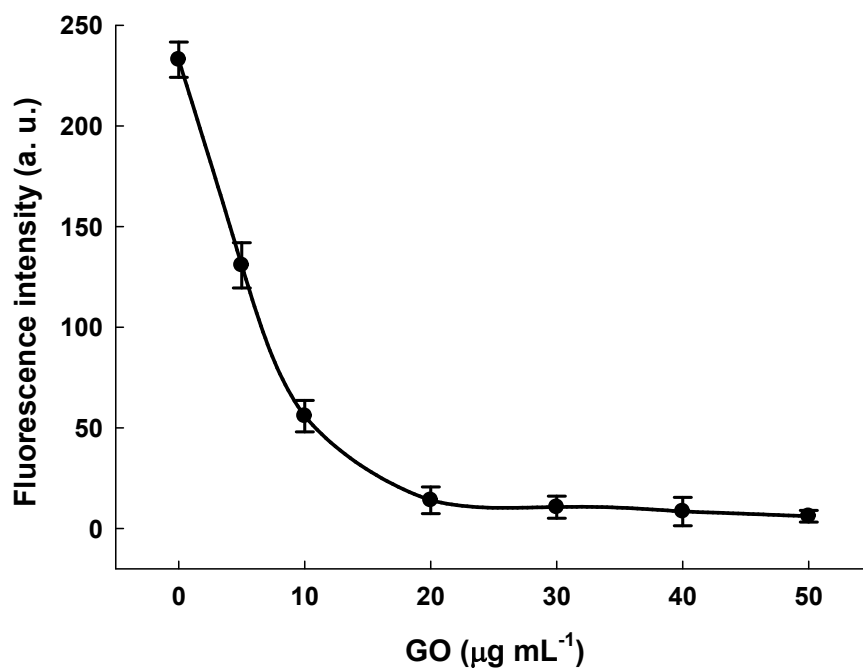
**Fig. S1** Different temperature effects on the fluorescence responses of the sensing system.

(a) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase ( $1 \text{ U } \mu\text{L}^{-1}$ ) + T4 PNK ( $0.2 \text{ U mL}^{-1}$ ) + ExoIII ( $0.1 \text{ U } \mu\text{L}^{-1}$ ) react at  $37 \text{ }^\circ\text{C}$ ;

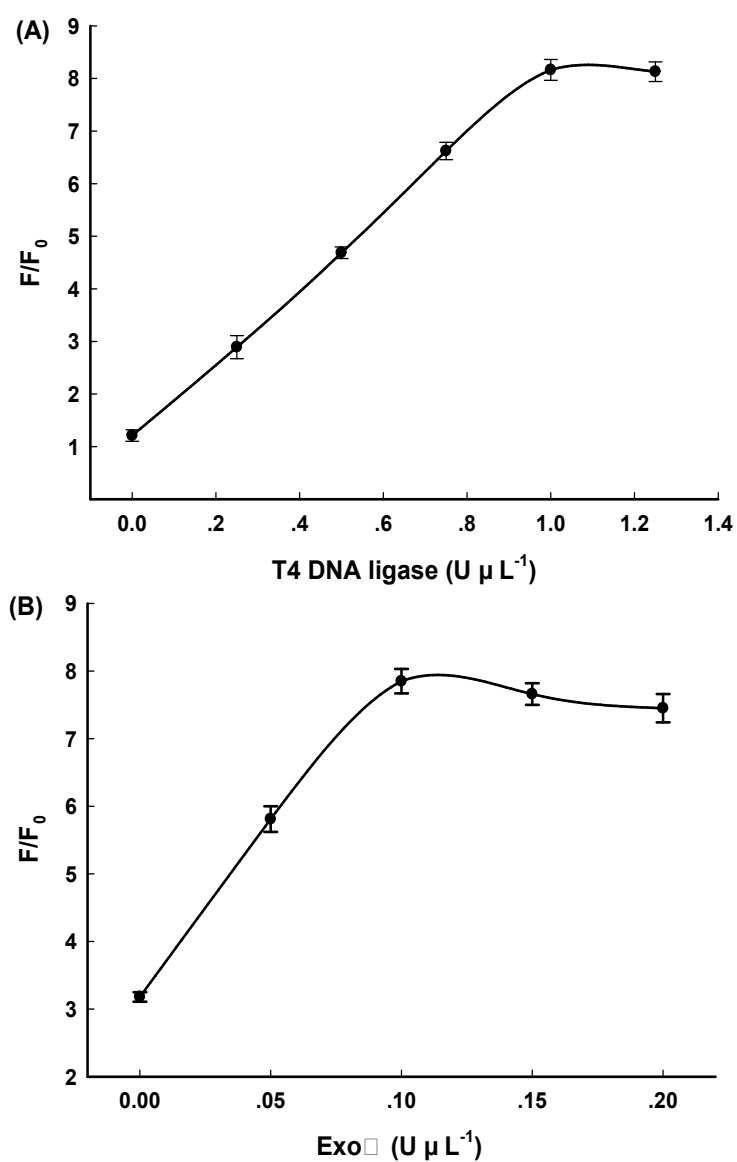
(b) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase ( $1 \text{ U } \mu\text{L}^{-1}$ ) + ExoIII ( $0.1 \text{ U } \mu\text{L}^{-1}$ ) react at  $37 \text{ }^\circ\text{C}$ ;

(c) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase ( $1 \text{ U } \mu\text{L}^{-1}$ ) + T4 PNK ( $0.2 \text{ U mL}^{-1}$ ) + ExoIII ( $0.1 \text{ U } \mu\text{L}^{-1}$ ) react at  $37 \text{ }^\circ\text{C}$ ;

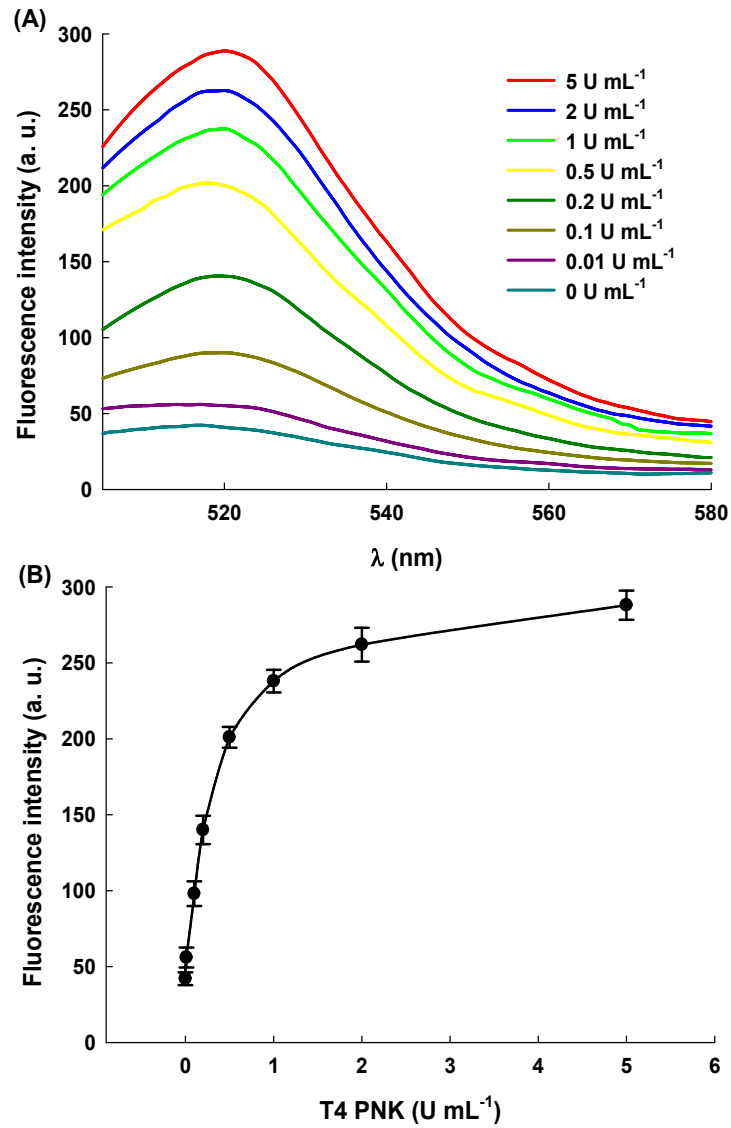
(d) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase ( $1 \text{ U } \mu\text{L}^{-1}$ ) + ExoIII ( $0.1 \text{ U } \mu\text{L}^{-1}$ ) react at  $37 \text{ }^\circ\text{C}$ .



**Fig. S2** Fluorescence intensities of 50 nM P3 in the presence of different concentrations of GO. Error bars were estimated from three replicate measurements.



**Fig. S3** The effect of the (A) T4 DNA ligase concentration and (B) ExoIII concentration on the fluorescence responses of the sensing system. The concentration of T4 PNK was 0.2 U mL<sup>-1</sup>. The descriptors F and F<sub>0</sub> are the fluorescence intensity in the presence and absence of target, respectively. Error bars were estimated from three replicate measurements.



**Fig. S4** (A) Fluorescence spectra responses of the sensing system in the presence of different concentrations of T4 PNK in diluted cell extracts (1%). (B) The relationship of the fluorescence enhancement with the T4 PNK concentration in diluted cell extracts (1%). Error bars were estimated from three replicate measurements.

**Table S1** Comparison of different fluorescent systems for PNK activity detection.

System	Linear Range (U mL <sup>-1</sup> )	Detection Limit (U mL <sup>-1</sup> )	Ref.
Molecular beacon	0.002 to 4.0	0.002	1
$\lambda$ -exonuclease reaction coupled perylene probe	0 to 0.08	0.003	2
$\lambda$ -exonuclease reaction coupled GO platform	0.05 to 10	0.05	3
$\lambda$ -exonuclease reaction coupled DNA-templated copper nanoparticle	0 to 3	0.49	4
Molecular beacon based GO platform	0 to 0.3	0.001	5
$\lambda$ -exonuclease reaction coupled molecular beacon	0.001 to 0.1	0.001	6
This work	0 to 0.2	0.003	

## References

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